Redesign a Non-Specific Endonuclease

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Recognition between proteins and DNA has been studied extensively because it plays the central role for all kinds of biological events, from DNA replication, transcription, recombination and repair to chromosome assembly and DNA degradation in apoptosis. It is therefore of crucial importance to elucidate the underlying principles that govern the binding selectivity between proteins and DNA. ColE7 is an *Escherichia coli* toxin, containing non-specific endonuclease activity. Here we use the nuclease domain of ColE7 (N-ColE7) as a model system to study the interactions between a protein and DNA molecules.

Based on computational methods, we constructed several nuclease-ColE7 mutants, K490R, D493N, D493Q and K497R, which were predicted to bind DNA with better affinity. The fluorescence steady-state assays show that D493N, D493Q and K497R indeed had increased DNA-binding affinity. The foot-printing assays further showed that D493N and D493Q digested DNA with a different sequence preference as compared to the wild-type enzyme. Moreover, the N-ColE7 mutant D493Q was co-crystallized with an 18-bp DNA and X-ray diffraction data were collected at NSRRC BL-13B1. The crystal structure of N-ColE7/DNA complex revealed that Q493 forms two hydrogen bonds with DNA. The extra hydrogen bond contributes to the mutant's higher DNA-binding affinity and altered sequence preference in cleavage. These results show that the combination of crystal structural analysis and computational methods is a useful device for generating a re-designed endonuclease with different properties in DNA binding and cleavage.

Figure a,b

Overall structure (Fig a) and electron density of close view around N-ColE7 D493Q in complex with 18mer DNA duplex. (Fig b)

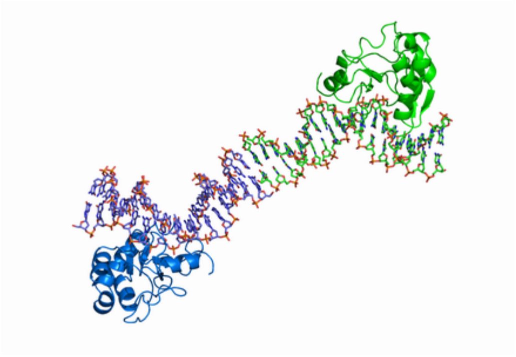


Figure a.

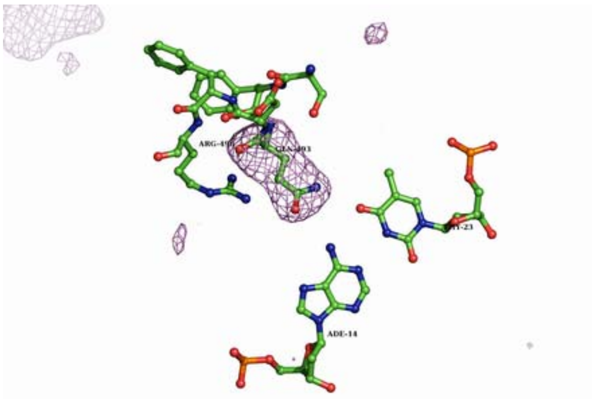
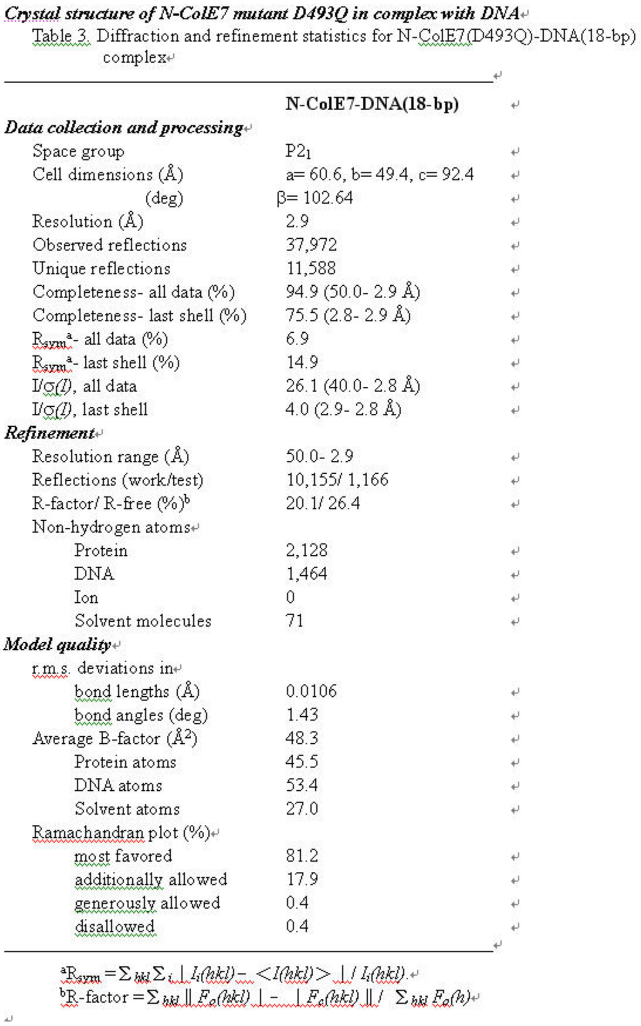


Figure b.



Crystal structure of N-ColE7 mutant D493Q in complex with DNA

Table 3. Diffraction and refinement statistics for N-ColE7(D493Q)-DNA(18-bp) complex

	N-ColE7-DNA(18-bp)
Data collection and processing	
Space group	$P2_1$
Cell dimensions (Å)	a= 60.6, b= 49.4, c= 92.4
(deg)	β= 102.64
Resolution (Å)	2.9
Observed reflections	37,972
Unique reflections	11,588
Completeness- all data (%)	94.9 (50.0- 2.9 Å)
Completeness- last shell (%)	75.5 (2.8- 2.9 Å)
$R_{sym}{}^{a}$- all data (%)	6.9
$R_{sym}{}^{a}$- last shell (%)	14.9
$I/\sigma(I)$, all data	26.1 (40.0- 2.8 Å)
$I/\sigma(I)$, last shell	4.0 (2.9- 2.8 Å)
Refinement	
Resolution range (Å)	50.0- 2.9
Reflections (work/test)	10,155/ 1,166
R-factor/ R-free (%)^{b}	20.1/ 26.4
Non-hydrogen atoms	
Protein	2,128
DNA	1,464
Ion	0
Solvent molecules	71
Model quality	
r.m.s. deviations in	
bond lengths (Å)	0.0106
bond angles (deg)	1.43
Average B-factor (Å^2)	48.3
Protein atoms	45.5
DNA atoms	53.4
Solvent atoms	27.0
Ramachandran plot (%)	
most favored	81.2
additionally allowed	17.9
generously allowed	0.4
disallowed	0.4

[a] $R_{sym} = \Sigma_{hkl} \Sigma_i \mid I_i(hkl) - \langle I(hkl) \rangle \mid / I_i(hkl)$.

[b] $\text{R-factor} = \Sigma_{hkl} \| F_o(hkl) \mid - \mid F_c(hkl) \| / \ \Sigma_{hkl} F_o(h)$